compared with susceptible flies and has similar sensitivity to that of the well-characterised 49r2b dimethoate-resistant strain, which has altered AChE activity.[2]

For permethrin, the 381bz strain has R/S of 615 at LD_{50} and 773 at LD_{95}, which can be partly synergized by PBO but only slightly synergised by DEF. This strain has a super-*kdr* allele of the Na-channel protein gene[3,4] and the pyrethroid resistance could be caused by a combination of the insensitive target site and metabolism due to GSTs and monooxgenases. The GST activity of the 381zb strain is significantly different from that of the susceptible WHO strain: DCNB activity is increased three-fold and CDNB activity is slightly elevated. We have not been able to link permethrin degradation directly to GST activity since it was neither an inducer nor an inhibitor of GST activity. The PNA monooxygenase activity of 381zb is *c.* two-fold greater than that of the WHO strain.

With the 594vb strain, the R/S with azamethiphos is 33 at LD_{50} and 370 at LD_{95}; at LD_{50} it is synergised by both PBO and DEF to R/S of 6 and 9, respectively. At the LD_{95} the R/S level changes to 2 and 14 for PBO and DEF, respectively. The reaction rate of uninhibited AChE in 594vb is very high compared to the three other strains tested, but it is strongly inhibited by azamethiphos. Involvement of AChE in azamethiphos resistance in the 594vb strain has yet to be demonstrated. The GST and PNA monooxygenase activities of the 594vb and WHO strains are similar.

Alterations of general esterase level of substrate specificity in relation to insecticide resistance have not been identified previously in Denmark, although they have been studied in at least 12 field-collected Danish strains.[5] We have measured esterase activity of the 381zb, 594vb and WHO strains using seven different esterase substrates (*p*-nitrophenylacetate, -propionate and -butyrate; α-naphthylacetate and -butyrate and β-naphthylacetate and -butyrate). There are subtle differences between males and females using some of the substrates and there are differences in the specific activity level with the different substrates. However, there is a high degree of correlation between the activity obtained with different esterase substrates, as shown by pairwise linear regressions. Thus, for screening of general elevated esterase activity, a single substrate will be sufficient.

The multiresistant 381zb strain contains many mechanisms which contribute to the observed resistance, like the well-characterised pyrethroid-resistant LPR strain where a combination of constitutively elevated P450 monooxygenase activity, insensitive target site and delayed penetration is responsible for the high level of resistance.[6] The 594vb strain, on the other hand, shows only weak indices of resistance with our current biochemical assay and needs to be bioassayed to detect the resistance.

This collection of baseline data for susceptible and Danish field-collected strains of houseflies will explain the implementation of a survey strategy which combines biological and biochemical assays although, to get a complete picture of insecticide resistance in natural populations, we need to include molecular biology, determination of the Na-channel protein gene and GABA-receptor gene alleles.

References

1. Keiding, J. & Jespersen, J. B., *Proc. Brit. Crop Prot. Conf.—Pests and Diseases* **2** (1986) 623–30.
2. Devonshire, A. L., Studies of the acetylcholinesterase from houseflies (*Musca domestica* L.) resistant and susceptible to organophosphorus insecticides. *Biochem. J.*, **149** (1975) 463–9.
3. Farnham, A. W., Murray, A. W. A., Sawicki, R. M., Denholm, I. & White, J. C., Characterization of the structure–activity relationship of *kdr* and two variants of super-*kdr* to pyrethroids in the house fly (*Musca domestica* L.), *Pestic. Sci.*, **19** (1987) 209–20.
4. Williamson, M. S., Martinez-Torres, D., Hick, C. A. & Devonshire, A. L., Identification of mutations in the housefly para-type sodium channel gene associated with known resistance (*kdr*) to pyrethroid insecticides. *Mol. Gen. Genet.*, **252** (1996) 51–60.
5. Sawicki, R. M., Devonshire, A. L., Farnham, A. W., O'Dell, K. E., Moores, G. D. & Denholm, I., Factors affecting resistance to insecticides in houseflies *Musca domestica* L. II. Close linkage on autosome 2 between an esterase and resistance to trichlorphon and pyrethroids. *Bull. Ent. Res.*, **74** (1984) 197–206.
6. Scott, J. G. & Georghiou, G. P., Mechanisms responsible for high levels of permethrin resistance in the house fly. *Pestic. Sci.*, **17** (1986) 195–206.

The Biochemical Detection of Insecticide Resistance in Danish Field Populations of the German Cockroach *Blattella germanica* (Blattellidae)

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Since the discontinuation of dieldrin use in the late 1970s due to resistance problems, the control of cockroaches in Denmark has relied on a strategy of alternate periods of chlorpyrifos/diazinon and permethrin/deltamethrin treatment. While resistance to pyrethroids arose within four years of their introduction, chlorpyrifos resistance sufficient to cause control problems has never been reported.[1] This is generally consistent with the experience in other parts of the world.

Earlier research into the basis of pyrethroid resistance in three Danish populations indicated that a range of resistance mechanisms were present.[2] We have found that pyrethroid resistance has fallen, but remains widespread and sufficient to cause control problems. Chlorpyrifos, however, remains an effective control agent in the field, with low resistance levels in all populations tested. Rust and Reierson[3] found evidence of chlorpyrifos-resistant German cockroach populations in the USA. This shows that such resistance can develop, and introduces the possibility that it may spread. With this in mind, we have established a program to monitor resistance in *Blattella germanica* L. from Denmark using an integrated approach involving both traditional in vivo and in vitro biochemical systems.

In this report we detail the results of a preliminary investigation involving four field strains, a laboratory susceptible strain and a laboratory resistant strain. All strains were bioassayed against insecticides commonly used in cockroach field control and assayed *in vitro* for enzyme systems commonly associated with resistance.

Six *B. germanica* strains maintained in culture in this laboratory (DPIL) were used in this investigation. Strain S, a laboratory susceptible strain, has been held at DPIL for more than 25 years. Strain A is multiresistant and has been maintained in culture at DPIL since 1989. Strains B, C, D and E were collected from sites in Copenhagen during 1996 and bred without selection through two to three generations in the laboratory.

Topical bioassays were carried out on adult male cockroaches, approximately 2·5 months old.[4] Results were analyzed by probit analysis to determine LD values.

Homogenates of individual adult male cockroaches were analysed using the model substrates α-naphthyl acetate, β-naphthyl acetate and *p*-nitrophenyl butyrate for esterase activity and dinitrochlorobenzene (DCNB) and dichloronitrobenzene (CDNB) for glutathione-*S*-transferase activity.[5] Sensitivity of AChE to inhibition was measured using azamethiphos and methomyl as substrates.[6] Statistical analysis was performed using SAS release 6·12.

Bioassay (detailed in Table 1) revealed a high level of permethrin resistance in all field strains (B, C, D and E). This is largely consistent with the results gained from a similar collection made in 1987/8.[1] Chlorpyrifos resistance, although much smaller, was significant in field strains B, C and D and greatest in laboratory resistant strain A.

Higher GST activity was recorded in all field strains than in either laboratory strain (Table 2). This difference was greatest of DCNB was used as the substrate.

For all three esterase substrates the mean activity was significantly higher in the three resistant field strains (B, C and D) than in the laboratory susceptible strain, but not in the OP-susceptible field strain (E) (Table 2). Analysis by Duncan's test for variability identified field strain B as being distinct from all other strains. Field strain E, which is not resistance to chlorpyrifos, has esterase activity very close to that of the laboratory susceptible strain.

The choice of inhibitors for the AChE study was made largely on the basis of availability. Strain B, which showed the highest esterase activity, showed no signs of AChE insensitivity (Table 2). Strain C, which also shows chlorpyrifos resistance, showed a significant level of insensitivity. The other resistant field strain (strain D) also showed signs of AChE insensitivity. It appears likely, therefore, that although target site insensitivity plays no role in the observed OP resistance in strain B, it may play a significant role in strains C and D. Confirmation of this initial conclusion with chlorpyrifos-oxon is planned.

The results in Table 3, in which the different strains are listed in decreasing order of resistance in each test, show a clear correlation between esterase activity in general and chlorpyrifos resistance. This is indicated not only by the mean activities but also by the groupings evolved using the Duncan test. It is therefore likely that

TABLE 1
Results from Topical Bioassays of Test Strains of *Blattella germanica*

	Chlorpyrifos			*Permethrin*			*Deltamethrin*		
Strain	LD_{50}[a]	LD_{95}	R/S-LD_{50}	LD_{50}	LD_{95}	R/S-LD_{50}	LD_{50}	LD_{95}	R/S-LD_{50}
S (DPIL-Sus)	0·014	0·031	1	0·014	0·064	1	0·00027	0·0012	1
A (HRDC)	0·11	0·48	7·6	0·021	0·044	1·4	0·001[b]	0·016[b]	3·7[b]
B (960301)	0·071	0·2	5·1	0·2	14	16	0·01	0·09	39
C (960318)	0·08[b]	0·32[b]	4[b]	0·67	1·9	47	0·01	0.05	44
D (960304)	0·08[b]	0.32[b]	4[b]	0.41	0·92	29			
E (960302)	0·015	0·025	1·1	0·29	0·87	20	0·006	0·04	23

[a] LD values are concentration of applied insecticide (% in acetone) giving 50 or 95% control. In all full bioassays 20 individuals were assayed using at least five concentrations giving between 0 and 100% mortality.
[b] Estimate based on preliminary data.

TABLE 2
Summary of Mean Esterase Activity, Glutathione-*S*-transferase activity and AChE Sensitivity Measured *in vitro* in 20 Adult Male Individuals for Each Strain[a]

Strain	*Permethrin* $R/S\text{-}LD_{50}$	*Chlorpyrifos* $R/S\text{-}LD_{50}$	*Mean GST activity* *CDNB*[b]	*DCNB*[b]	*Mean esterase activity* *αNA*[c]	*βNA*[c]	*p-NPB*[b]	*Mean AChE sensitivity*[c] *Methomyl*	*Azamethiphos*
S (DPIL-Sus)	1	1	49	1·4	0·19	0·23	85	22	27
A (HRDC)	1·4	7·6	79	1·9	0·23	0·3	121	26	25
B (960301)	16	5·1	80	2·9	0·23	0·32	122	21	30
C (960318)	47	4	83	2·9	0·23	0·28	115	28	32
D (960304)	29	4	74	2·4	0·21	0·28	105	25	33
E (960302)	20	1·1	66	2·7	0·18	0·26	97	23	26

[a] The same set of homogenates was used for each assay.
[b] nmol min^{-1} mg^{-1}.
[c] μmol min^{-1} mg^{-1}.
[d] % activity remaining after treatment.

TABLE 3
Relative Magnitude of Measured Resistance, Mean Enzyme Activities and Mean Levels of AChE Insensitivity for the Six Strains Tested[a]

	$R/S\text{-}LD_{50}$		*Mean GST activity*		*Mean esterase activity*			*Mean AChE insensitivity*	
	Permethrin	*Chlorpyrifos*	*CDNB*	*DCNB*	*αNA*	*βNA*	*p-NPB*	*Methomyl*	*Azamethiphos*
Highest	C	A	C^{a}	C^{a}	A^{a}	B^{a}	B^{a}	C^{a}	D^{a}
	D	B	B^{ab}	B^{a}	B^{a}	A^{ab}	A^{a}	A^{ab}	C^{ab}
	E	C	A^{ab}	E^{a}	C^{a}	C^{bc}	C^{ab}	D^{b}	B^{b}
	B	D	D^{bc}	D^{b}	D^{ab}	D^{bc}	D^{bc}	E^{c}	S^{c}
	A	E	E^{c}	A^{c}	E^{b}	E^{c}	E^{c}	S^{cd}	E^{c}
Lowest	S	S	S^{d}	S^{d}	S^{b}	S^{d}	S^{d}	B^{d}	A^{c}

[a] Each column is arranged independently according to magnitude. Superscript characters represent Duncan groupings determined by SAS using individual data.

esterases play a significant role in resistance to chlorpyrifos.

On the other hand, there appears to be no such correlation in respect to AChE activity, although, as mentioned, insensitivity may play a role in resistance in strains C and D. Based on the data collected, there appears to be no correlation between pyrethroid resistance and the activities measured.

Although resistance to pyrethroids remains high in Danish *B. germanica*, it is of far less significance than resistance, or the possibility of resistance, to chlorpyrifos, since chlorpyrifos has, for many years, remained Denmark's only guarantee against control failure. The existence of individuals with high esterase activity and/or AChE insensitivity in field populations showing a degree of resistance is cause for concern and worthy of further investigation. It is our intention to collect cockroaches from a greater number of sites over the coming months and to look in greater depth at the mechanisms responsible for any resistance detected. We also intend to confirm the apparent close link between esterase activity and chlorpyrifos resistance, and to establish which mechanism or combination of mechanisms is responsible for the high pyrethroid resistance.

References

1. Jensen, K.-M. V., Insecticide resistance in *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from food-producing establishments in Denmark. *Proc. 1st Internat. Conf. on Insect Pests in the Urban Environment*. ed. K. B. Wildey & W. H. Robinson, 1993, 135–9.
2. Hemingway, J., Small, G. J. & Monro, A. G., Possible mechanisms of organophosphorus and carbamate insecticide resistance in German cockroaches (Dictyoptera: Blattellidae) from different geographical areas. *J. Econ. Entomol.*, **86** (1993) 1623–30.
3. Rust, M. R. & Reierson, D. A., Chlorpyriphos resistance in German cockroaches (Dictyoptera: Blattellidae) from restaurants. *J. Econ. Entomol.*, **84** (1991) 736–40.
4. Anon. *Danish Pest Infestation Laboratory Annual Report* (1987) 58.
5. Yu, S. J. & Nguyen, S. N., Detection and biochemical characterization of insecticide resistance in the Diamondback Moth. *Pestic. Biochem. Physiol.*, **44** (1992) 74–81.
6. Ellman, G. L., Courtney, D. K., Andres, V. & Featherstone, R. M., A new and rapid colorimetric determination of acetylcholinesterase activity *Biochem. Pharmacol.*, **7** (1961) 88–95.